

SUPPORTING INFORMATION

Figure S1

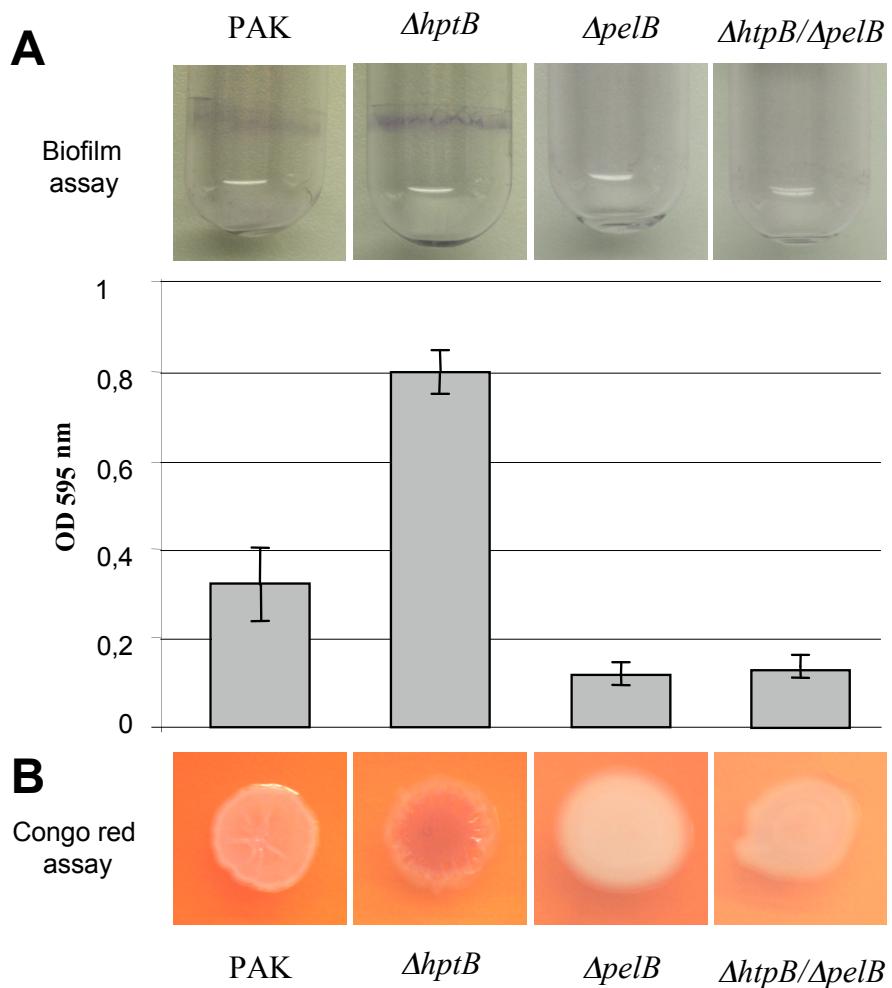


Fig. S1. Effect of *pelB* deletion gene on *htpB* phenotypes.

A. Glass tube assay showing biofilm formation (upper part). Quantification of the crystal violet-stained adherence ring formed in the glass tube (lower part). Each experiment was repeated three times. The error bars indicate standard deviations. The name of the tested strain is indicated above each panel.

B. Bacterial colony staining on Congo Red-containing agar plates. The name of strains used is indicated under each panel.

Figure S2

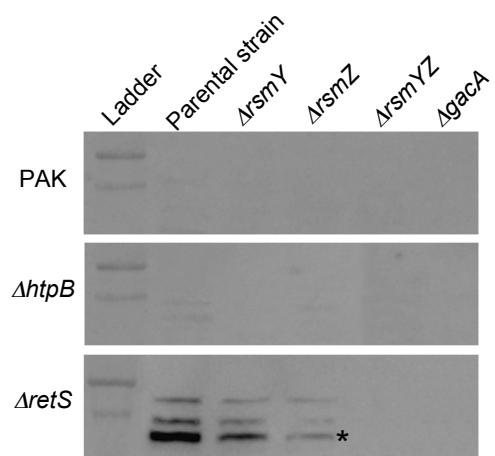


Fig. S2. Production of the type VI secretion component VgrG1. Immunodetection of VgrG1 (indicated by an asterisk) on whole cell extracts of strains PAK, PAK Δ hptB and PAK Δ retS indicated as parental strain). VgrG1 production was also tested in each parental strain carrying an additional rsmY, rsmZ, rsmYZ or gacA mutation.

Figure S3

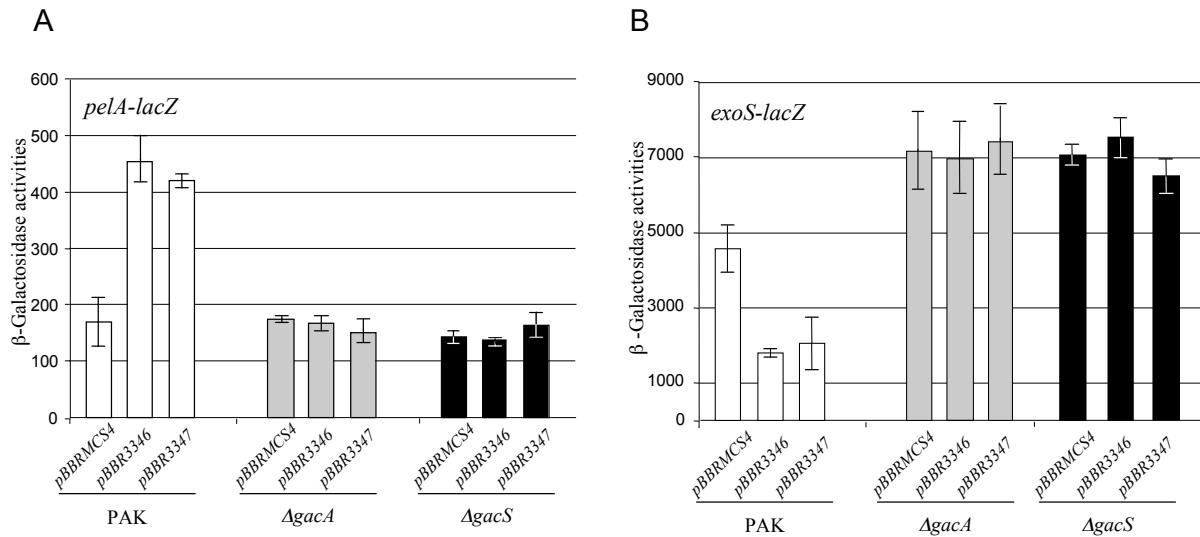


Fig. S3. Expression of *lacZ* transcriptional fusion in PAK, PAK $\Delta gacA$ or PAK $\Delta gacS$ strains overexpressing *PA3346* (pBBR3346) or *PA3347* (pBBR3347) or containing the cloning vector pBBRMCS4. Activity was recorded after 4 hours growth.

A. Activity of the *pelA-lacZ* transcriptional fusion.

B. Activity of the *exoS-lacZ* transcriptional fusion (carried on pSB307). White bars correspond to PAK, grey bars to PAK $\Delta gacA$ and black bars to PAK $\Delta gacS$. The plasmid contained by each of these strains is indicated under the corresponding bar.

β-galactosidase activities are expressed in Miller units. Values are averages of at least three independent experiments.

Figure S4

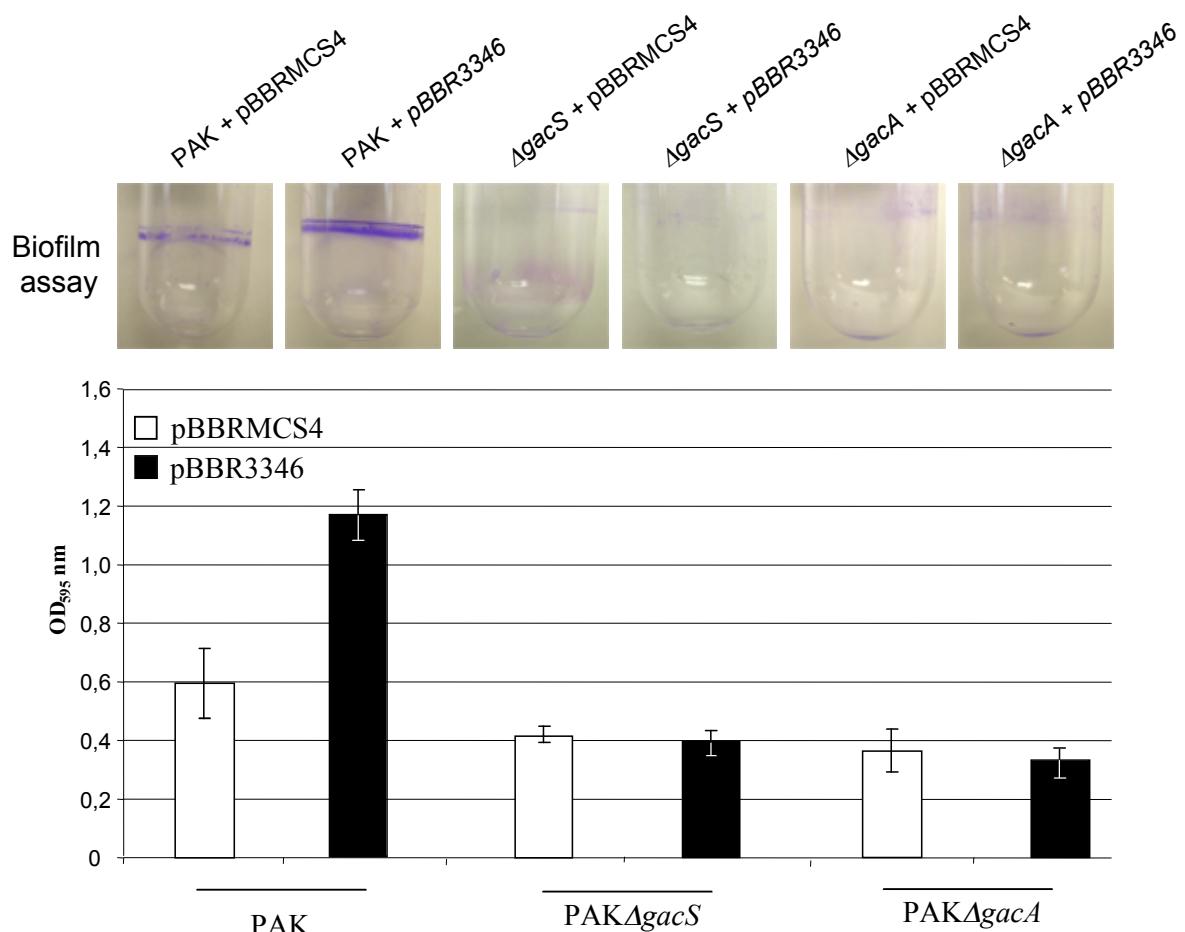


Fig. S4. Influence of *PA3346* overexpression in PAK, PAK $\Delta gacA$ or PAK $\Delta gacS$ strains on biofilm formation.

A. Glass tube assay showing biofilm formation. The name of the tested strains is indicated above each panel with *PA3346* expressed from the pBBR3346 plasmid whereas pBBRMCS4 is the cloning vector.

B. Quantification of the adherence ring formed in the glass tube. Each experiment was repeated three times. The error bars indicate standard deviations. The name of the strains used is indicated under each bar. Filled bars correspond to strains carrying pBBR3346 whereas open bars correspond to strains carrying pBBRMCS4. The pBBR3346 allowed overexpression of the *PA3346* gene cloned into the pBBRMCS4 vector.

Figure S5

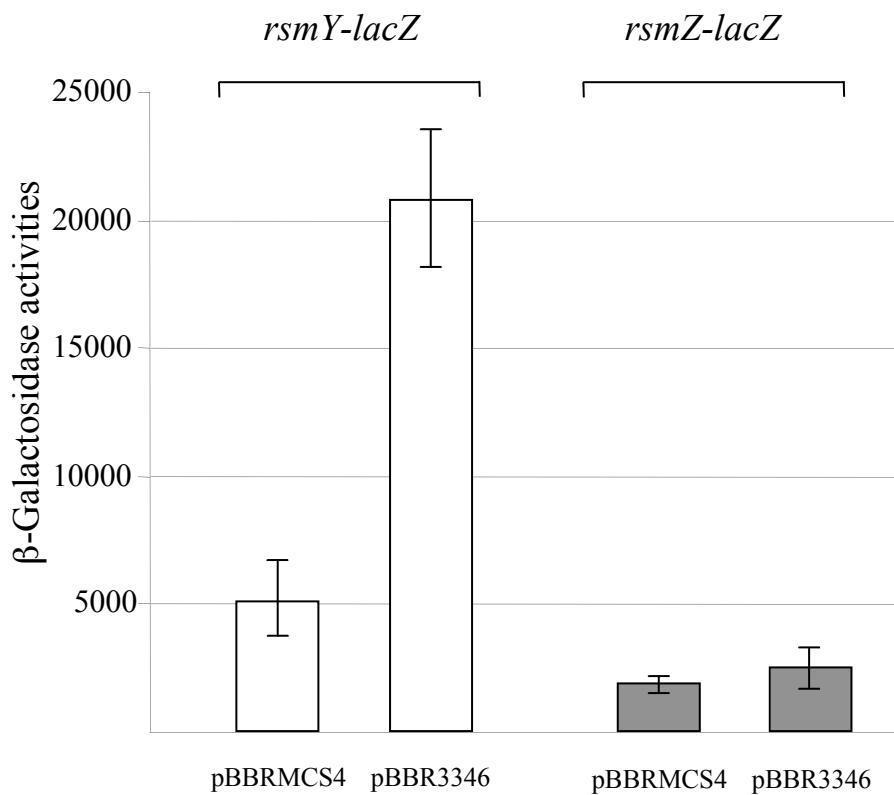


Fig. S5. Expression of the *rsmY* and *rsmZ* genes in *P. aeruginosa* strains overexpressing PA3346 or not. Activity of the *rsmY-lacZ* (open bars) and *rsmZ-lacZ* (filled bars) transcriptional fusion in PAK containing pBBRMCS4 or the derivative plasmid pBBR3346 overexpressing *PA3346*, was recorded after 4 hours of growth. β -galactosidase activities are expressed in Miller units. Values are averages of at least three independent experiments.

SUPPORTING INFORMATION

Table S1. Strains and plasmids used in this study.

Strains/Plasmids	Relevant characteristics ^a	Source/Reference
Strains		
<i>P. aeruginosa</i>		
PAK	Wild type	D. Bradley
PAK Δ retS	Deletion of retS in PAK	Goodman <i>et al.</i> (2004)
PAK Δ hptB	Deletion of hptB in PAK	This study
PAK Δ PA3346	Deletion of PA3346 in PAK	This study
PAK Δ PA3347	Deletion of PA3347 in PAK	This study
PAK Δ gacA	Deletion of gacA in PAK	This study
PAK Δ gacS	Deletion of gacS in PAK	This study
PAK Δ rsmY	Deletion of rsmY in PAK	This study
PAK Δ rsmZ	Deletion of rsmZ in PAK	This study
PAK Δ pelB	Deletion of pelB in PAK	Vasseur <i>et al.</i> (2005)
PAK Δ hptB Δ pelB	Deletion of pelB in PAK Δ hptB	This study
PAK Δ hptB Δ gacA	Deletion of gacA in PAK Δ hptB	This study
PAK Δ hptB Δ gacS	Deletion of gacS in PAK Δ hptB	This study
PAK Δ hptB Δ rsmY	Deletion of rsmY in PAK Δ hptB	This study
PAK Δ hptB Δ rsmZ	Deletion of rsmZ in PAK Δ hptB	This study
PAK Δ hptB Δ rsmY Δ rsmZ	Deletion of rsmZ in PAK Δ hptB Δ rsmY	This study
PAK Δ hptB Δ PA3346	Deletion of PA3346 in PAK Δ hptB	This study
PAK Δ hptB Δ PA3347	Deletion of PA3347 in PAK Δ hptB	This study
PAK Δ retS Δ gacA	Deletion of gacA in PAK Δ retS	This study
PAK Δ retS Δ gacS	Deletion of gacS in PAK Δ retS	This study
PAK Δ retS Δ rsmY	Deletion of rsmY in PAK Δ retS	This study
PAK Δ retS Δ rsmZ	Deletion of rsmZ in PAK Δ retS	This study
PAK Δ retS Δ rsmY Δ rsmZ	Deletion of rsmZ in PAK Δ retS Δ rsmY	This study
PAK Δ retS Δ PA3346	Deletion of PA3346 in PAK Δ retS	This study
PAK Δ retS Δ PA3347	Deletion of PA3347 in PAK Δ retS	This study
<i>E. coli</i>		
TG1	supE Δ (lac-proAB) thi hsdR Δ 5 (F' traD36 rpoA ⁺ B ⁺ lacI ^q Z Δ M15) F'(lacI ^q Tn10(Tet ^R)) mrcA Δ (mrr-hsdRMS-mcrBC) Φ 80 lacZ Δ M15 Δ lacX74 recA1 araD139 Δ (ara-leu)7697 galU galK rpsL(Str ^R) endA1 nupG	Lab collection
TOP10F'		Invitrogen
DHM1	Reporter strain for two-hybrid system (F ⁻ , glnV44(AS), recA1, endA1, gyrA96 (Nal ^r), thi1, hsdR17, spoT1, rfbD1, cya) Propagation of pKNG101 plasmid and derivatives; Δ (ara-leu) araD Δ lacX74 galE galK phoA20 thi-1 rpsE rpoB argE(Am) recA1 Rf ^r (λ pir)	Karimova <i>et al.</i> (2000)
CC118 λ pir		Lab collection
Plasmids		

pCR2.1	ColE1, f1 <i>ori</i> , Ap ^R , Km ^R	Invitrogen
pRK2013	Tra ⁺ Mob ⁺ Km ^R	Lab collection
pMP220	Broad-host-range <i>lacZ</i> transcriptional fusion, Tc ^r	Lab collection
ppelA-lacZ	pMP220 plasmid carrying a <i>pelA-lacZ</i> transcriptional fusion	Vasseur <i>et al.</i> (2005)
pSB307	pMP220 plasmid carrying a <i>exoS-lacZ</i> transcriptional fusion	Bleves <i>et al.</i> (2005)
pMP220rsmZ-lacZ	pMP220 plasmid carrying a <i>rsmZ-lacZ</i> transcriptional fusion	This study
pMP220rsmY-lacZ	pMP220 plasmid carrying a <i>rsmY-lacZ</i> transcriptional fusion	This study
pET-Dest42 TM	pET-Dest42 TM , destination vector, LR recombination, V5 epitope and hexahistidine region (V5H6), ColE1, pT7lac, Ap ^R , Cm ^R , ccdB	Invitrogen
pETvgrG1	V5H6 tagged <i>vgrG1</i> cloned in pET-Dest42 TM	This study
pUCP18	Broad host range plasmid, Ap ^R	Lab collection
pUCPhptB	pUCP18 carrying the <i>hptB</i> ORF	This study
pBBRMCS4	Broad host range plasmid, Ap ^R	Kovach <i>et al.</i> (1995)
pBBR3346	pBBRMCS4 carrying the PA3346 ORF	This study
pBBR3347	pBBRMCS4 carrying the PA3347 ORF	This study
pKT25	Two-hybrid plasmid, cyaAT25 fusion, Km ^R	Karimova <i>et al.</i> (2000)
pUT18C	Two-hybrid plasmid, cyaAT18 fusion, Ap ^R	Karimova <i>et al.</i> (2000)
pKT25-hptB	Two-hybrid plasmid containing cyaAT25– <i>hptB</i> fusion	This study
pKT25-PP2C	Two-hybrid plasmid containing cyaAT25–PA3346 PP2C domain fusion	This study
pUT18C-3346D	Two-hybrid plasmid containing cyaAT18–PA3346 D2 receiver domain fusion	This study
pUT18C-3347	Two-hybrid plasmid containing cyaAT18–PA3347 fusion	This study
pKNG101	Suicide vector in <i>P. aeruginosa</i> ; <i>SacB</i> St ^r	Kaniga <i>et al.</i> (1991)
pKNGΔhptB	Mutator plasmid for <i>hptB</i> deletion	This study
pKNGΔgacA	Mutator plasmid for <i>gacA</i> deletion	This study
pKNGΔgacS	Mutator plasmid for <i>gacS</i> deletion	This study
pKNGΔrsmY	Mutator plasmid for <i>rsmY</i> deletion	This study
pKNGΔrsmZ	Mutator plasmid for <i>rsmZ</i> deletion	This study
pKNGΔPA3346	Mutator plasmid for <i>PA3346</i> deletion	This study
pKNGΔPA3347	Mutator plasmid for <i>PA3347</i> deletion	This study
pKNGmamb3063	Mutator plasmid for <i>pelB</i> deletion	Vasseur <i>et al.</i> (2005)

^aAp^R ampicillin, Str^R streptomycin, Km^R kanamycin and Tc^R tetracycline

Table S2. Genes whose expression varies in *hptB* and *retS* background^a.

Gene expression varies both in PAK Δ <i>hptB</i> and PAK Δ <i>retS</i> mutant		Gene expression varies in PAK Δ <i>retS</i> mutant		
Genes ^b	Ratio in PAK Δ <i>hptB</i>	Ratio in PAK Δ <i>retS</i>	Genes ^c	Ratio in PAK Δ <i>retS</i>
<i>pelB</i>	3,19	4,46	PA0084	22,82
<i>pelA</i>	3,44	4,45	PA0085	9,12
<i>exoS</i>	-4,44	-53,7	PA0070	8,26
<i>exoT</i>	-6,14	-5,34	PA0083	7,66
<i>exoY</i>	-7,41	-16,01	PA0086	6,97
<i>exsA</i>	-5,1	-5,52	<i>arcC</i>	6,58
<i>exsD</i>	-5,84	-4,53	PA0087	5,88
PA1697 (PscN ATPase)	-2,29	-3,21	PA0089	5,63
PA2189	-2,32	-6,38	PA0126	5,05
PA3842 (Probable ExoS chaperone)	-2,23	-11,18	PA3729	4,78
PA3844	-2,60	-4,76	<i>psl4</i>	4,11
<i>pcrD</i>	-2,89	-2,92	PA3716	4,05
<i>pcrH</i>	-4,23	-14,98	PA0563	4,01
<i>popB</i>	-6,32	-41,24	PA5033	3,99
<i>popD</i>	-5,34	-10,5	PA1658	3,98
<i>popN</i>	-6,74	-5,39	PA3484	3,78
<i>pscF</i>	-3,18	-4,61	PA2537	3,73
<i>pscJ</i>	-5,19	-6,07	PA0095	3,70
<i>pscL</i>	-6,03	-4,65	<i>arcB</i>	3,58
			PA2204	3,39
			PA5136	3,25
			PA1068	3,10
			PA0277	3,08
			PA2581	3,04
			<i>oprC</i>	3,04
			PA0078	3,04
			<i>norC</i>	2,99
			PA4317	2,97
			PA4487	2,96
			PA0659	2,87
			PA3661	2,82
			PA0628	2,79
			PA0320	2,78
			PA3021	2,76
			PA0859	2,75
			PA0730	2,74
			PA1494	2,72
			<i>sbp</i>	2,71
			PA4491	2,67
			PA4495	2,61
			PA0045	2,57
			<i>pslG</i>	2,55
			PA1202	2,49
			PA0625	2,46
			<i>mety</i>	2,43
			PA4318	2,43
			PA3727	2,41

PA5530	2,39
<i>oprH</i>	2,36
PA0620	2,33
PA0201	2,32
PA1510	2,29
PA4321	2,21
PA3450	2,21
PA0434	2,19
PA1791	2,17
PA3022	2,14
PA1555	2,13
PA0141	2,12
PA4746	2,12
<i>pykF</i>	2,11
<i>nosZ</i>	2,07
PA0456	2,07
PA5178	2,05
<i>nrdB</i>	2,04
PA3931	2,02
PA2536	2,02
<i>phoP</i>	2,01
PA1132	2,00
PA0169	2,00
<i>gbuA</i>	-29,12
<i>exsB</i>	-5,49
<i>hpD</i>	-5,26
PA2776	-4,76
<i>bkdA2</i>	-4,56
PA3840	-4,47
<i>bkdA1</i>	-4,40
<i>htpG</i>	-4,22
<i>dada</i>	-3,85
PA0779	-3,84
PA1418	-3,76
<i>spuC</i>	-3,76
PA1742	-3,60
<i>gabD</i>	-3,59
<i>groEL</i>	-3,59
<i>putP</i>	-3,51
<i>pilV</i>	-3,50
PA5312	-3,49
<i>pscK</i>	-3,46
PA1416	-3,28
<i>pilE</i>	-3,22
<i>dnaK</i>	-3,21
<i>pilX</i>	-3,21
PA1342	-3,16
<i>pscD</i>	-3,15
PA4506	-3,04
PA0296	-3,00
PA2040	-2,87
PA2016	-2,83
PA4505	-2,76
PA4504	-2,73

PA5436	-2,68
PA4503	-2,63
<i>fimU</i>	-2,62
PA0604	-2,60
<i>prpC</i>	-2,57
<i>pilW</i>	-2,57
<i>hslU</i>	-2,54
PA4918	-2,48
<i>fahA</i>	-2,47
<i>gnyH</i>	-2,38
<i>spuB</i>	-2,38
<i>braC</i>	-2,32
<i>phhA</i>	-2,28
PA4078	-2,26

^aGenes down-regulated in the mutant versus the parental strain are displayed on green background, those up-regulated are on orange background.

^b*exoS, exoT, exoY, exsA, exsD, PA1697, pcrD, pcrH, popB, popD, popN, pscF, pscJ, pscL* are type III secretion genes.

^cType VI secretion genes are written with thick characters.

Table S3. Oligonucleotides used in this study.

Name	Mutation/Cloning	Restriction site	Sequence 5'→ 3' ^a
BupA	<i>hptB</i> deletion	<i>Xba</i> I	GCT <u>CTAGAAACCC</u> CTCAAGCATTCAACCCGCTG
BupB	<i>hptB</i> deletion	<i>Eco</i> RI	TCC <u>GAATTCTATCGCTGAGGTTTCG</u> CCTTCC
BloA	<i>hptB</i> deletion	<i>Eco</i> RI	ATA <u>GAATT</u> CGGACATTGATCGCTCCCTGAAGTC
BloB	<i>hptB</i> deletion	<i>Spe</i> I	GG <u>ACTAGTGGTGC</u> CAGGTAGAGCAGCTTGATCT
B5	<i>hptB</i> deletion		GAAGATCGGATATTCTGTT
B6	<i>hptB</i> deletion		ACGATGCGGTAGAAGATGTT
Bup	<i>hptB</i> complementation		TTAAC <u>GTTATGGTAGGGCATCGGAA</u> GT
Bdown	<i>hptB</i> complementation		AAGGATCCGACGAAAACCTCAGCGATAG
3346up	<i>PA3346</i> overexpression	<i>Eco</i> RI	CG <u>GAATTCTGAACACACG</u> TCTCCGTCG
3346down	<i>PA3346</i> overexpression	<i>Bam</i> HI	CGGG <u>ATCCGCTGGTAAAGGAAGG</u> CCTTGG
3347up	<i>PA3347</i> overexpression	<i>Eco</i> RI	CG <u>GAATTCACTGCTTACATGATTCAAG</u> CC
3347down	<i>PA3347</i> overexpression	<i>Bam</i> HI	CGGG <u>ATCCTCAGCTGATCTGAACA</u> ACTGC
UAgacA	<i>gacA</i> deletion	<i>Bam</i> HI	CG <u>CGGATCCTAGTGCTGATCGGTGACGCC</u>
LAGacA	<i>gacA</i> deletion	<i>Eco</i> RI	AA <u>AGAATTCAATCACGCTGCACCTGCTCG</u>
UBgacA	<i>gacA</i> deletion	<i>Eco</i> RI	AA <u>AGAATT</u> CTAGATGAGCGCCGTTTCGA
LBgacA	<i>gacA</i> deletion	<i>Spe</i> I	GG <u>ACTAGTTCCTCGAGGAACATCACCG</u>
UAgacS	<i>gacS</i> deletion	<i>Bam</i> HI	CGGG <u>ATCCGATCATGCTGGTATGACCG</u>
LAgacS	<i>gacS</i> deletion	<i>Eco</i> RI	CG <u>GAATTCCACACGCTCTCCGTCGAGCC</u>
UBgacS	<i>gacS</i> deletion	<i>Eco</i> RI	CG <u>GAATTCGAACTCTGACCATGCGCATCC</u>
LBgacS	<i>gacS</i> deletion	<i>Spe</i> I	GG <u>ACTAGTCCGGTATTGATCAGCATCGC</u>
UArsmY	<i>rsmY</i> deletion	<i>Bam</i> HI	CG <u>CGGATCCAACCGAACAGCTGGCTGG</u>
LArsmY	<i>rsmY</i> deletion	<i>Xho</i> I	CC <u>GCTCGAGATTACGCATCTCGAGGG</u>
UBrsmY	<i>rsmY</i> deletion	<i>Xho</i> I	CC <u>GCTCGAGTTATTGCCGAGGAAAACCG</u>
LBrsmY	<i>rsmY</i> deletion	<i>Spe</i> I	GG <u>ACTAGTCCGGAAATCGACATCGAGCG</u>
UArsmZ	<i>rsmZ</i> deletion	<i>Bam</i> HI	CG <u>CGGATCCTAGACGTCTCTGGTCCG</u>
LArsmZ	<i>rsmZ</i> deletion	<i>Xho</i> I	CC <u>GCTCGAGCCTGCCGTTTACTCGTCGC</u>
UBrsmZ	<i>rsmZ</i> deletion	<i>Xho</i> I	CC <u>GCTCGAGCCTGCCGTTTACTCGTCGC</u>
LBrsmZ	<i>rsmZ</i> deletion	<i>Spe</i> I	GG <u>ACTAGTGCACAAGCTGCTAGAATCGC</u>
UAPA3346	<i>PA3346</i> deletion	<i>Bam</i> HI	GC <u>GGATCCGCTGGGACGGCAGGTGGGAC</u>
LAPA3346	<i>PA3346</i> deletion	<i>Xho</i> I	CC <u>GCTCGAGGGATTACCATCAGCTGATCTTG</u>
UBPA3346	<i>PA3346</i> deletion	<i>Xho</i> I	CC <u>GCTCGAGTTCAAACAGGAACGTCAGCGC</u>
LBPA3346	<i>PA3346</i> deletion	<i>Spe</i> I	GG <u>ACTAGTTGCAGGAAGGCCAGCATGCGCG</u>
UAPA3347	<i>PA3347</i> deletion	<i>Bam</i> HI	CG <u>CGGATCCCCGGAGCGTCTGCAACGCTAT</u>
LAPA3347	<i>PA3347</i> deletion	<i>Xho</i> I	CC <u>GCTCGAGGCCATGGAAGTCTCTGGTA</u>
UBPA3347	<i>PA3347</i> deletion	<i>Xho</i> I	CC <u>GCTCGAGGTTGTTCAAAGATCAGCTGAGA</u>
LBPA33467	<i>PA3347</i> deletion	<i>Spe</i> I	GG <u>ACTAGTCGCCGAGCAGCACGTGCATGCC</u>
PrsmY1	<i>rsmY</i> promoter	<i>Eco</i> RI	CG <u>GAATTCAAGGCTCGCGATGATGAGG</u>
PrsmY2	<i>rsmY</i> promoter	<i>Kpn</i> I	GG <u>GGTACCTTGGCGCTTCCTGCGCAATGTCC</u>
PrsmZ1	<i>rsmZ</i> promoter	<i>Eco</i> RI	CG <u>GAATTCCCTAGACCCACTGAAGACC</u>
PrsmZ2	<i>rsmZ</i> promoter	<i>Kpn</i> I	GG <u>GGTACCATCCTCGGGTTGCGTGTCC</u>
UDHhptB	HptB for two hybrid	<i>Xba</i> I	GCT <u>CTAGAGCGAATGTCCCGCCGATCTCGATCGT</u> G

DDHhtpB	HtpB for two hybrid	<i>KpnI</i>	<u>GGGCCGGTACCTGTCGCCGGAAAGGACGAAACCTCAGC</u>
UDH3347	PA3347 for two hybrid	<i>XbaI</i>	<u>GCTCTAGAGATGCCATCACTGCGCTGCC</u>
DDH3347	PA3347 for two hybrid	<i>KpnI</i>	<u>GGGGTACCTCAGCTGATCTGAACAAC</u> TGC
UDHPP2C	PP2C of PA3346 for two hybrid	<i>XbaI</i>	<u>GCTCTAGACAACGTGCGTACCTGCAATCG</u>
DDHPP2C	PP2C of PA3346 for two hybrid	<i>SacI</i>	<u>CCGAGCTCCGACGAAAGCGCGATTATGCCTGAGG</u>
UDHD3346	Receiver domain of PA3346 for two hybrid	<i>XbaI</i>	<u>GCTCTAGATGAGATGAATCCCCGGCGG</u>
DDHD3346	Receiver domain of PA3346 for two hybrid	<i>EcoRI</i>	<u>AACGGAATTCTCACGGCGAGAAATACTGGTT</u> CG
L1R1	Microarray construction		ACAAGTTGTACAAAAAAGCAGGCT
L2R2	Microarray Construction		ACCACTTGACAAGAAAGCTGGGT

^aRestriction site sequence is underlined.

SUPPORTING INFORMATION

References

Kovach, M.E., Elzer, P.H., Hill, D.S., Robertson, G.T., Farris, M.A., Roop, R.M., and Peterson, K.M. (1995). Four new derivatives of the broad-host-range cloning vector pBBR1MCS, carrying different antibiotic-resistance cassettes. *Gene* **166**, 175–176.